Remarks

The Amendments

Claim 15 has been amended to recite that the mixture is of "primer pairs," in place of "primers." Claim 15 has also been amended to recite that the ratio of the primer pairs in the claimed mixture is described, in part, by the length "in base pairs" of the amplicons amplified in a multiplex amplification reaction. Claim 15 has further been amended to recite that the primer pairs in the mixture are present at a calculated ratio of "molar concentrations" in place of "concentrations." These amendments are supported by the specification at Tables 2 and 3, which disclose the concentration of primer pairs that amplify an amplicon, the base pair length of amplicons to be amplified in a multiplex amplification reaction, and the molar concentration of primers that amplify the amplicons.

New claims 25-32 have been added. These claims are similar to originally filed claims 17-24, but in independent claim format.

The specification has been amended to recite the newly assigned U.S. patent number of priority application 09/989,441.

These amendments introduce no new matter.

The Objections to the Specification

The Office Action objects to the specification on two separate grounds. Each is discussed below.

(1) The Office Action objects to the specification because applicants allegedly filed a substitute specification on December 23, 2003 but did not provide a clean copy of the substitute

specification. Office Action at page 2, lines 3-5. Applicants, however, did not file a substitute specification under 37 CFR § 1.125 on December 23, 2003. Applicants filed the subject application and a preliminary amendment on December 23, 2003. The preliminary amendment filed December 23, 2003 provides instructions to amend two paragraphs of the specification: "Please amend the paragraph at page 1, lines 4-6 with the following paragraph," (page 2, line 2) and "Please amend the paragraph at page 6, lines 4-23 as follows" (page 2, line 8). The amendment also contains a marked up reproduction of each indicated paragraph under its corresponding instruction. The two instructions and their corresponding two marked-up specification paragraphs are the required elements for making amendments to the specification under 37 CFR § 1.121(b)(1). Rule 37 CFR § 1.121 does not require that applicants provide a clean copy of the amended specification or specification paragraphs. Thus applicants' amendment under 37 CFR § 1.121(b)(1) is proper and should be entered.

Applicants did not file a substitute specification under 37 CFR § 1.125 on December 23, 2003 and respectfully request withdrawal of this objection.

(2) The Office Action objects to the specification as informal because it does not provide the newly assigned U.S. patent number for priority application 09/989,441. The specification has been amended to disclose that application serial number 09/989,441 issued as U.S. patent number 6,673,579. Applicants respectfully request withdrawal of this objection.

The Rejection of Claims 15-24 Under 35 U.S.C. § 112, second paragraph

Claims 15-24 stand rejected under 35 U.S.C. § 112, second paragraph. Applicants respectfully traverse.

The Office Action asserts that claims 15-24 are vague and indefinite because no unit is provided for the length of the amplicon (L_A) and the length of the longest amplicon (L_L) in the recited equation $C_A=C_L (L_A \div L_L)^2$. To expedite prosecution, applicants have amended claim 15 to recite that L_A and L_L are the length in "base pairs" for amplicon A and the longest amplicon, respectively. Thus, the unit length for each of the amplicons recited in claim 15 and dependent claims 16-24 is clearly recited.

Applicants respectfully request withdrawal of this rejection.

The Rejection of Claims 15-22 Under 35 U.S.C. § 102(e)

Claims 15-22 have been rejected under 35 U.S.C. § 102(e) as anticipated by Diamandis *et al.* (U.S. Patent Number 5,552,283; "Diamandis"). Applicants respectfully traverse.

Claim 15 is the only independent claim of the rejected claim set. Claim 15 is directed to a mixture of primer pairs for performing multiplex polymerase chain reaction. Each of the primer pairs is present in the mixture at a predetermined ratio to each other. The ratio of the molar concentrations of the primer pairs is described by the following equation:

$$C_A=C_L (L_A \div L_L)^2.$$

C_A is the molar concentration of primers for an amplicon A. C_L is the molar concentration of primers for the longest amplicon. L_A is the length in base pairs of the amplicon A. L_L is the length in base pairs of the longest amplicon.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Brothers v. Union Oil Company of California*, 814 F.2d 628 (Fed. Cir. 1987). Diamandis does not expressly

or inherently describe each and every element set forth in claim 15.

Diamandis teaches methods for detecting *p53* mutations. One method detects *p53* insertion and deletion mutations by analyzing the length of each *p53* gene exon. Column 5, lines 37-44. The method of detecting these mutations includes a step of performing a first multiplex amplification reaction to amplify *p53* exons 1, 3, 4, 5, 6, 9, 10, and 11 (column 11, lines 59-61) and a second multiplex amplification reaction to amplify *p53* exons 2 and 8 (column 12, lines 50-51). Contrary to the assertion of the Patent Office, the ratio of the concentrations of the primers used in each of the first and the second multiplex amplification reactions is not described by $C_A=C_L(L_A \div L_L)^2$ as recited in claim 15.

Diamandis teaches using 150 ng of each primer to amplify each p53 exon in the first and the second multiplex amplification reactions. Diamandis teaches, "Each 50 microliter multiplexed PCR reaction contains 0.5 micrograms genomic DNA, 150 ng of each primer. . . ." Column 11, lines 44-46. Thus, Diamandis does not expressly teach a mixture of primer pairs in which the concentrations of the primer pairs present in the mixture is described by $C_A=C_L(L_A \div L_L)^2$.

Diamandis also does not inherently teach a mixture of primer pairs for performing a multiplex chain reaction in which the concentrations of the primer pairs are present at a ratio as determined in claim 15. The use of 150 ng each primer in the first multiplex amplification reaction yields a ratio of the molar concentrations of primer pairs as follows, where the longest exon (10) is arbitrarily set at a concentration of 1:

1.07 (1): 1.14 (3): 0.96 (4): 1.13 (5): 1.13 (6): 1.07 (9): 1.00 (10): 1.08 (11).

The formula recited in claim 15, however, provides a different molar ratio for the concentration

of primer pair in the first multiplex amplification reaction. The formula recited in claim 15 yields a molar ratio for the concentration of primer pair as follows, where the longest exon (10) is again set at a concentration of 1:

0.72 (1): 0.17 (3): 0.96 (4): 0.47 (5): 0.40 (6): 0.29 (9): 1.00 (10): 0.43 (11).

The last two columns of the following table provide a side-by-side comparison of these ratios and demonstrate how they were obtained. See also Matsuzaki declaration, attached.

Exon Number	Amplicon Size (bp)	Primers Used to Amplify Each Exon	Molecular Weight of Each Primer	Concentration of each primer when 150 ng is used in a 50 µl reaction (nM) (Diamandis)	Concentration of primers for amplification of each exon using 150 ng each primer in a 50 µl reaction (nM) (Diamandis)	Molar Ratio of Primers Based on use of 150 ng each primer used in a 50 µl reaction (Diamandis)	Molar ratio determined by formula recited in claim 15: $C_A = \frac{C_L(L_A \div L_L)}{2}$
1	331	5'-CGGATTACTTGCCCTTACTTGTCA-3'	7270.7	412.6	940.3	1.07	0.72
		5'-CCCCAGCCCCAGCGATTTT-3'	5685.7	527.6			
3	162	5'-CATGGGACTGACTTTCTGCT-3'	6100	491.8	1005.5	1.14	0.17
		5'-GGACGGCAACGCCGACTGT-3'	5839.8	513.7			
4	382	5'-CTGGTCCTCTGACTGCTCTTTTCA-3'	7237.7	414.5	848.8	0.96	0.96
		5'-AAAGAAATGCAGGGGGATACGG-3'	6907.5	434.3			
5	268	5'-TGTTCACTTGTGCCCTGACT-3'	6050.9	495.8	995.3	1.13	0.47
		5'-CAGCCCTGTCGTCTCTCCAG-3'	6005.9	499.5			
6	247	5'-CTGGGGCTGGAGAGACGACA-3'	6233.1	481.3	995.6	1.13	0.40
		5'-GGAGGGCCACTGACAACCA-3'	5832.8	514.3			
9	209	5'-GCGGTGGAGGAGACCAAGG-3'	5968.9	502.6	944.4	1.07	0.29

Exon Number	Amplicon Size (bp)	Primers Used to Amplify Each Exon	Molecular Weight of Each Primer	Concentration of each primer when 150 ng is used in a 50 µl reaction (nM) (Diamandis)	Concentration of primers for amplification of each exon using 150 ng each primer in a 50 µl reaction (nM) (Diamandis)	Molar Ratio of Primers Based on use of 150 ng each primer used in a 50 µl reaction (Diamandis)	Molar ratio determined by formula recited in claim 15: $C_A = C_L(L_A \div L_L)_2$
		5'-AACGGCATTTTGAGTGTTAGAC-3'	6790.5	441.8			
10	390	5'-TGATCCGTCATAAAGTCAAACAA-3'	7025.6	427.0	882.2	1.00	1.00
		5'-GTGGAGGCAAGAATGTGGTTA-3'	6591.3	455.1			
11	256	5'-GGCACAGACCCTCTCACTCAT-3'	6312.1	475.3	949.0	1.08	0.43
		5'-TGCTTCTGACGCACACCTATT-3'	6333.1	473.7			

Thus, the ratio of primer pairs disclosed by Diamandis for use in the first multiplex amplification reaction is not the same as that recited by the subject claims.

The use of 150 ng of each primer in the second multiplex amplification reaction taught by Diamandis (column 12, lines 50-51) also does not yield the same molar ratio of the concentration of primer pairs as that calculated by the equation described in claim 15. The use of 150 ng each primer in the second multiplex amplification reaction yields a molar ratio of the concentration of primer pairs as follows: `1.17:1.00` for the primer sets that amplify exons 2 and 8, respectively. The present invention, however, requires a molar ratio of `0.67:1.00` for the same primer pairs. See Matsuzaki declaration.

Exon Number	Amplicon Size (bp)	Primers Used to Amplify Each Exon	Molecular Weight of Each Primer	Concentration of each Primer when 150 ng is used in a 50 µl reaction (nM) (Diamandis)	Concentration of primers for Amplification of each Exon using 150 ng each primer in a 50 µl reaction (nM) (Diamandis)	Molar Ratio of Primers Based on use of 150 ng each primer used in a 50 µl reaction (Diamandis)	Molar ratio determined by formula recited in claim 15: $C_A = \frac{C_L(L_A \div L_L)}{2}$
2	261	5'-ACCCAGGGTTGGAAGCGTCT-3'	6159.0	487.1	929.1	1.17	0.67
		5'-GACAAGAGCAGAAAGTCAGTCC-3'	6787.5	442.0			
8	320	5'-GACAAGGGTGGTTGGGAGTAGATG-3'	7579.0	395.8	794.1	1.00	1.00
		5'-GCAAGGAAAGGTGATAAAAGTGAA-3'	7533.0	398.2			

Thus, the ratio of primer pairs disclosed by Diamandis for use in the second multiplex amplification reaction is also not the same as that recited by the subject claims. Therefore, Diamandis does not inherently teach a mixture of primer pairs for performing multiplex polymerase chain reaction in which the ratio of the molar concentrations of the primer pairs is described by $C_A=C_L(L_A \div L_L)^2$.

The Diamandis reference does not expressly or inherently teach a mixture of primer pairs for performing multiplex polymerase chain reaction in which the molar ratio of primer pairs in the reaction is described by $C_A = C_L (L_A \div L_L)^2$. Thus, Diamandis does not teach each and every element of claim 15. Applicants respectfully request withdrawal of this rejection to claim 15.

Claims 16-24 depend from claim 15 and thus also require that the primer pairs in the mixture of primer pairs for performing multiplex polymerase chain reaction be present at a molar

ratio described by $C_A = C_L (L_A \div L_L)^2$. As indicated above, Diamandis does not expressly or inherently teach this element of claim 15 and thus also does not expressly or inherently teach this element of claims 16-24. Applicants respectfully request withdrawal of the rejection to claims 16-24.

The Rejection of Claims 23 and 24 Under 35 U.S.C. § 103

Claims 23 and 24 are rejected under 35 U.S.C. § 103(a) as obvious over Diamandis. Applicants respectfully traverse.

The U.S. Patent and Trademark Office bears the initial burden of establishing a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531 (Fed. Cir. 1993). The *prima facie* case requires three showings:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Manual of Patent Examining Procedure, 8th ed., § 2142. The Patent Office has not made a *prima facie* case of obviousness because Diamandis does not teach or suggest all the elements of the rejected claims.

Claims 23 and 24 depend from claim 15 and further require that the mixture of primer pairs comprise at least 18 and 20 primers, respectively. As discussed above, claim 15 requires that each of the primer pairs be present at a predetermined ratio to each other. The ratio is described by: $C_A=C_L (L_A \div L_L)^2$. Diamandis does not teach performing a multiplex

amplification reaction in which each of the primer pairs in the reaction is present at a molar ratio as determined in claim 15. Diamandis teaches first and second multiplex amplification reactions of the *p53* gene in which the primers are present in the amount of 150 ng each. See discussion, above, and Matsuzaki declaration.

Furthermore, Diamandis does not suggest a mixture of primer pairs for performing multiplex polymerase chain reaction that are present at a predetermined ratio to each other as described by $C_A=C_L (L_A \div L_L)^2$. Diamandis does not teach any method of determining a ratio of primer pairs to be used to perform multiplex polymerase chain reaction. Diamandis merely teaches using primers in the amount of 150 ng to perform multiplex polymerase chain reaction. Thus, Diamandis does not suggest a mixture of primer pairs at a predetermined ratio as described by claim 15.

Diamandis does not teach or suggest that the ratio of the molar concentrations of primer pairs present in a mixture of primers for performing multiplex polymerase chain reaction be determined by $C_A=C_L (L_A \div L_L)^2$ as recited in dependent claims 23 and 24. Thus Diamandis does not teach or suggest all the elements recited in claims 23 and claim 24. The *prima facie* case of obviousness must fail.

Applicants respectfully request withdrawal of this rejection.

Respectfully submitted,

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